

Effects of electrolyzed hydrogen water ingestion during endurance exercise in a heated environment on body fluid balance and exercise performance

Hiroto Ito^a, Shigeru Kabayama^b, and Kazushige Goto^a

^aGraduate School of Sport and Health Science, Ritsumeikan University, Shiga, Japan; ^bNihon Trim Co., Ltd, Osaka, Japan

ABSTRACT

Electrolyzed hydrogen water (EHW) is generated at a cathode. It contains many hydrogen molecules with high alkaline properties. The physiological effects of ingesting EHW during endurance exercise are unclear. The purpose of this study was to determine the effects of ingesting EHW during endurance exercise in a heated environment on body fluid balance and exercise performance. Twelve triathletes (20.0 ± 1.3 years, 171 ± 6 cm, 60.6 ± 3.9 kg, $\dot{V}O_{2\max}$ 67.1 ± 3.8 mL/kg/min) performed pedaling exercise for 60 min at 65% of $\dot{V}O_{2\max}$ consuming either purified water (CON trial) or EHW (EHW trial) and then conducted an incremental pedaling test. Blood parameters, tissue temperature, and respiratory variables were determined during 60 min of exercise. The time to exhaustion (TTE) during the incremental pedaling test was also determined. Body weights were 1.1 ± 0.4 kg lower after exercise, with no significant differences between trials. Plasma volume and serum osmolality and blood sodium and potassium concentrations significantly changed with exercise, but no significant differences were observed between trials. The pH, blood lactate and bicarbonate concentrations, and changes in skin and muscle temperature did not significantly differ between the two trials. Energy expenditure during exercise was significantly ($P = 0.04$) lower in the EHW trial (13.2 ± 0.5 kcal/min) than in the CON trial (13.7 ± 0.4 kcal/min). TTE did not significantly differ between the trials. In conclusion, EHW ingestion during endurance exercise in a heated environment decreased energy expenditure but did not affect body fluid balance or exercise performance.

Abbreviations: CON: control trial; CV: coefficient of variation; EHW: electrolyzed hydrogen water; HR: heart rate; RPE: rating of perceived exertion; SE: standard error; TP: total protein; TTE: time to exhaustion

ARTICLE HISTORY

Received 9 November 2019
Revised 3 February 2020
Accepted 4 February 2020

KEYWORDS

Electrolyzed hydrogen water; dehydration; endurance exercise; fluid balance; energy expenditure

Introduction

Electrolyzed hydrogen water (EHW) is generated in an electrolysis cell near the cathode, and is highly alkaline and rich in dissolved molecular hydrogen [1]. Xue et al. [2] reported that ingesting EHW attenuated gastric injury [2]. However, no data is available about the effects of EHW ingestion on body fluid balance during exercise.

Endurance exercise in a heated environment increases sweat loss [3], which reduces plasma volume, leading to increased blood viscosity [4]. Walking for 2 h at 35% of maximal oxygen uptake ($\dot{V}O_{2\max}$) increased plasma viscosity [5]. Greater blood viscosity and dehydration also increase the cutaneous vascular resistance, blood pressure, and heart rate (HR) [6], decreasing performance during prolonged exercise.

Logan-Sprenger [4] reported that an 11% decrease in plasma volume, increased HR, the core temperature, and rating of perceived exertion (RPE), and increased the time (by 13%) to complete a cycling time trial. Exercising in a heated environment increases body temperature. Skin temperature is affected by the environmental temperature, and an elevated skin temperature is associated with increased sweating and decreased plasma volume [7]. This physiological response plays an important role in sweat evaporation and maintaining body temperature during exercise in the heat. However, the attenuated sweat loss with dehydration impairs thermoregulatory function and exercise performance [8]. A sweat loss greater than 2% reduces exercise performance [9]. Therefore, maintaining the fluid balance during exercise in

CONTACT Kazushige Goto  kagoto@fc.ritsumei.ac.jp

This article has been republished with minor changes. These changes do not impact the academic content of the article.

Founding: NIHON TRIM CO., LTD.

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

a heated environment would be valuable for preventing heat illness and improving exercise performance. Weidman [10] reported that EHW ingestion attenuated the exercise-induced increase in blood viscosity. Therefore, we consider that ingesting EHW may attenuate the plasma volume loss during early phase of prolonged exercise in a heated environment by promoting fluid absorption.

Other studies have reported that hydrogen molecules are antioxidants with negative redox potential [3]. In sports, the ingestion of EHW is thought to attenuate exercise-induced muscle fatigue and impaired muscle function. Aoki et al. [11] demonstrated that ingesting EHW significantly attenuated the exercise-induced blood lactate elevation [11]. In addition, ingesting EHW for 14 days increased the blood pH from baseline [12]. The mechanism for fatigue during exercise is complicated, but exercise-induced acidosis (i.e., lower pH in muscle and blood) is thought to reduce exercise performance [13].

As described above, majority of the previous studies have examined the influence of dehydration during endurance exercise in a heated environment [8]. However, the impact of different types of water (e.g., water with various components) on the plasma volume shift during exercise has not been fully elucidated. Therefore, this study determined the effects of ingesting EHW during endurance exercise in a heated environment on body fluid balance and exercise performance. We hypothesized that EHW ingestion during the exercise would attenuate exercise-induced acidification and dehydration and the performance decrement compared to ingesting pure water.

Methods

Participants

Twelve triathletes [mean \pm standard deviation (SD); age 20.0 ± 1.3 years, height 171.0 ± 6.0 cm, body mass 60.6 ± 3.9 kg, $\dot{V}O_{2\max}$ 67.1 ± 3.8 mL/kg/min] participated in the present study. All subjects belonged to the triathlon club at the same university, and they had been specifically training for triathlons 5 days a week (about 3 h a day). Exclusion criteria were a history of an inflammatory condition and

musculoskeletal disorders. Smokers and individuals taking antioxidant supplements were also excluded. The subjects were instructed to maintain their normal diet, physical activity level, and training throughout the study. They gave informed consent after being informed of the purpose and risks associated with the study. This study was approved by ethics committee of Ritsumeikan University, Japan.

Study design

The subjects visited the laboratory three times over the study period. At the first visit, they completed an incremental pedaling test on a cycle ergometer (Power Max V-III; Konami, Kanagawa, Japan) to determine their individual $\dot{V}O_{2\max}$. The initial workload was set at 100 W for 2 min (90 rpm), and the load was increased by 30 W every 2 min until exhaustion. Respiratory gases were collected by a breath-by-breath method using an automatic gas analyzer (AE300 S; Minato Medical Science, Tokyo, Japan). On the second and third visits, the subjects visited the laboratory following an overnight fast. They then performed an exercise trial at the same time of each day. The two exercise trials consisted of exercise while drinking purified water (CON trial) or electrolyzed hydrogen water (EHW trial). These two trials were conducted at least 3 days apart, and the order of the trials was randomized.

The exercise in each trial consisted of 60 min of pedaling on a cycling ergometer (Power Max V-III; Konami, Kanagawa, Japan) at 65% of $\dot{V}O_{2\max}$. After completing 60 min of pedaling at a fixed workload, an incremental pedaling test until exhaustion was performed to evaluate their endurance exercise performance. A 5 min rest was provided between the completion of the 60 min of pedaling and the start of the incremental pedaling test. All exercises were conducted at 32°C and 50% relative humidity (Figure 1). The average workload was 217 ± 23 W. The subjects drank 2.0 mL/kg of either purified water or EHW every 15 min based on the previous study [14].

Preparation of EHW

The EHW was made using a commercial generator (Trim Ion Grace; Nihon Trim, Osaka, Japan) 30 min before the first drink on exercise days. The pH was

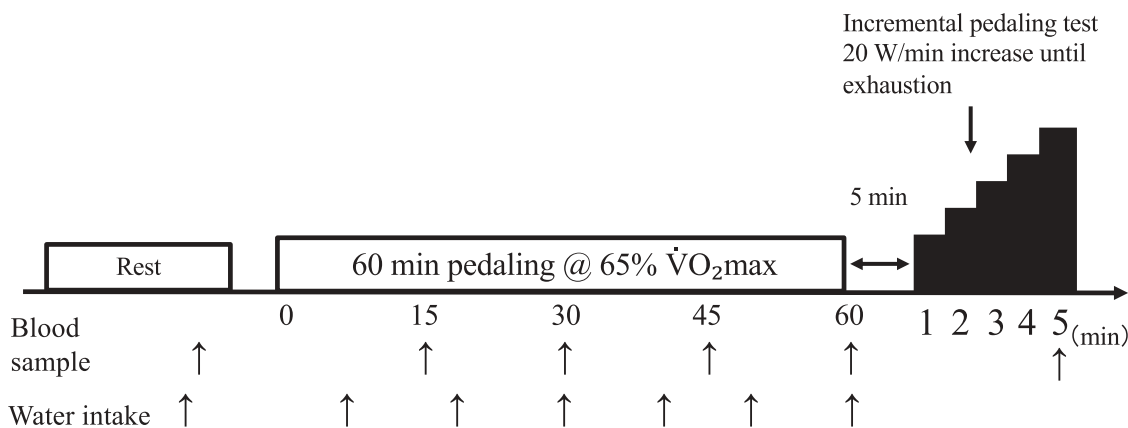


Figure 1. Experimental overview.

9.7 ± 0.2 for EHW (alkaline property) and 7.4 ± 0.1 for the pure water, which was produced using the same generator. During the experiment, the water was stored in a refrigerator on the exercise days.

Measures

Body weight and body composition

Before and after completing all exercises, body weight and body composition (e.g., muscle mass, fat mass, and body fluid volume) were evaluated using a bio-impedance technique (InBody 770; InBody, Cerritos, USA).

Body temperature (skin and muscle)

Skin temperature was measured at four sites (chest, arm, thigh, and calf) using probe type thermometers (NK543 Nikkiso Tokyo, Japan) and the mean skin temperature was calculated using the following equation [15]:

$$\text{Mean skin temperature} = 0.3 \times (\text{chest} + \text{arm}) \\ + 0.2 \times (\text{thigh} + \text{calf}).$$

The temperature of the rectus femoris muscle was evaluated noninvasively with a thermistor (XX-CM210PD1; Terumo, Tokyo, Japan) connected to a core thermometer (Core Temp CM210; Terumo Tokyo, Japan), according to the manufacturer's instructions. The thermometer assessed the core temperature 1 cm below the skin. Skin temperature was measured halfway between the nipple and clavicle for the chest, 60% of the distance between the acromion and

elbow for the arm, halfway between the greater trochanter and knee for the thigh, and 30% of the distance between the knee and lateral malleolus for the calf. The rectus femoris muscle temperature was evaluated halfway between the greater trochanter and knee. Data for skin and muscle temperatures were collected every 2 s.

Respiratory variables

Breath gas samples for $\dot{V}O_2$, $\dot{V}CO_2$, and the respiratory exchange ratio (RER) were collected and analyzed using an automatic gas analyzer breath-by-breath (AE300 s; MINATO Medical Science, Osaka, Japan). Energy expenditure was calculated using the equation [16]. The respiratory variables were collected at 25–30 and 55–60 min during the 60 min pedaling exercise. The values obtained breath-by-breath were averaged every 30 s.

Heart rate, scores of subjective feelings

HR and the RPE for respiration and legs were monitored every 10 min during the 60 min of pedaling. HR was measured continuously using a wireless HR monitor (RCX5; Polar, Finland, Japan). The RPE for respiration and legs was recorded using a modified Borg scale ranging from 0 (none at all) to 10 (maximal exertion) [17]. Thermal sensation and feeling thirsty or bloated during the exercise were evaluated every 10 min using a visual analog scale.

Blood sampling and analyses

On exercise days, the subjects visited the laboratory following an overnight fast. They were instructed to

finish dinner before 22:00 the previous night. After arriving at the laboratory, a cannula was inserted into an antebrachial vein (after a 30 min rest). Blood samples were collected six times: before exercise (after a 30 min rest); every 15 min during the 60 min pedal; and immediately after the incremental pedaling test. The blood samples were used to measure blood glucose, lactate, serum total protein (TP), and albumin and glycerol concentrations. Blood gas and acid–base balance parameters (e.g., blood pH and lactate concentration) and blood hemoglobin and hematocrit were also evaluated. Serum samples were obtained by centrifugation (3,000 rpm, 10 min, 4°C) and stored at –60°C until analyses. Blood glucose and lactate concentrations were measured using an automatic glucose analyzer (Free Style; Nipro Corporation, Osaka, Japan) and lactate analyzer (Lactate Pro; Arkray, Kyoto, Japan). Serum TP and albumin concentrations were measured at a clinical laboratory (SRL, Tokyo, Japan). Serum glycerol concentrations were determined in duplicate using a commercial kit (Cayman Chemical, Ann Arbor, MI, USA). The intra-assay coefficient of variation (CV) for measurement for glycerol concentration was 3.6%.

Blood gas parameters (e.g., blood pH, hemoglobin, hematocrit, and bicarbonate ion concentration) were evaluated using a gas analyzer (OPTI CCA-TS2; Sysmex, Hyogo, Japan).

Time to exhaustion

After finishing 60 min of continuous pedaling, the subjects started an incremental pedaling test until exhaustion to evaluate their endurance performance. The initial workload was set as 65% of $\dot{V}O_{2\max}$ and it was increased by 20 W every 2 min until exhaustion. When the subjects could not maintain the prescribed pedaling rate of 80 rpm for five successive seconds, the exercise was terminated [18].

Statistical analysis

Data are expressed as the mean \pm standard error (SE). Time course changes in RPE, HR, and the blood variables were initially analyzed using two-way analysis of variance (ANOVA) (trial \times time) with repeated measures. When the ANOVA revealed a significant interaction or

main effect, the Turkey–Kramer *post hoc* test was used to assess the difference. The differences in respiratory variables, body weight, and time to exhaustion (TTE) were analyzed using paired *t*-tests.

Results

The amount of water consumed in each trial averaged 926 ± 13 mL during exercise.

Reduction in body weight

Table 1 shows the change in body weight between before and immediately after exercise. Although body weight significantly decreased with exercise in CON trial (-1.1 ± 0.5 kg) and EHW trial (-1.1 ± 0.6 kg) (main effect of time, $P < 0.01$), the exercise-induced reduction did not significantly differ between the two trials (interaction, $P > 0.05$).

Tissue (skin and muscle) temperature

Figure 2 shows the muscle and skin temperatures during exercise. Skin temperatures increased rapidly during exercise (main effect of time, $P < 0.01$). Average skin temperature among the four sites (arm, chest, thigh, and calf) was $35.5 \pm 0.3^\circ\text{C}$ in the CON trial and $35.5 \pm 0.6^\circ\text{C}$ in the EHW trial. There was no significant interaction (trial \times time) ($P = 0.22$) or main effect of the trial ($P = 0.76$). Muscle temperature was significantly elevated during exercise in both trials (main effect of time, $P < 0.05$). However, these values did not significantly differ between the two trials (interaction, $P = 0.60$, main effect of trial, $P = 0.17$).

Heart rate, scores of subjective feelings

The average HR during 60 min of exercise was 163 ± 8 bpm in CON and 163 ± 10 bpm in the EHW trial, and did not significantly differ

Table 1. Exercise-induced absolute and relative changes in body weight.

Trial	Absolute change (kg)	Relative change (%)	Fluid intake (ml)
CON	-1.1 ± 0.5	-1.7 ± 0.7	926 ± 13
EHW	-1.1 ± 0.4	-1.8 ± 0.6	926 ± 13

Values are means \pm SD ($n = 12$)

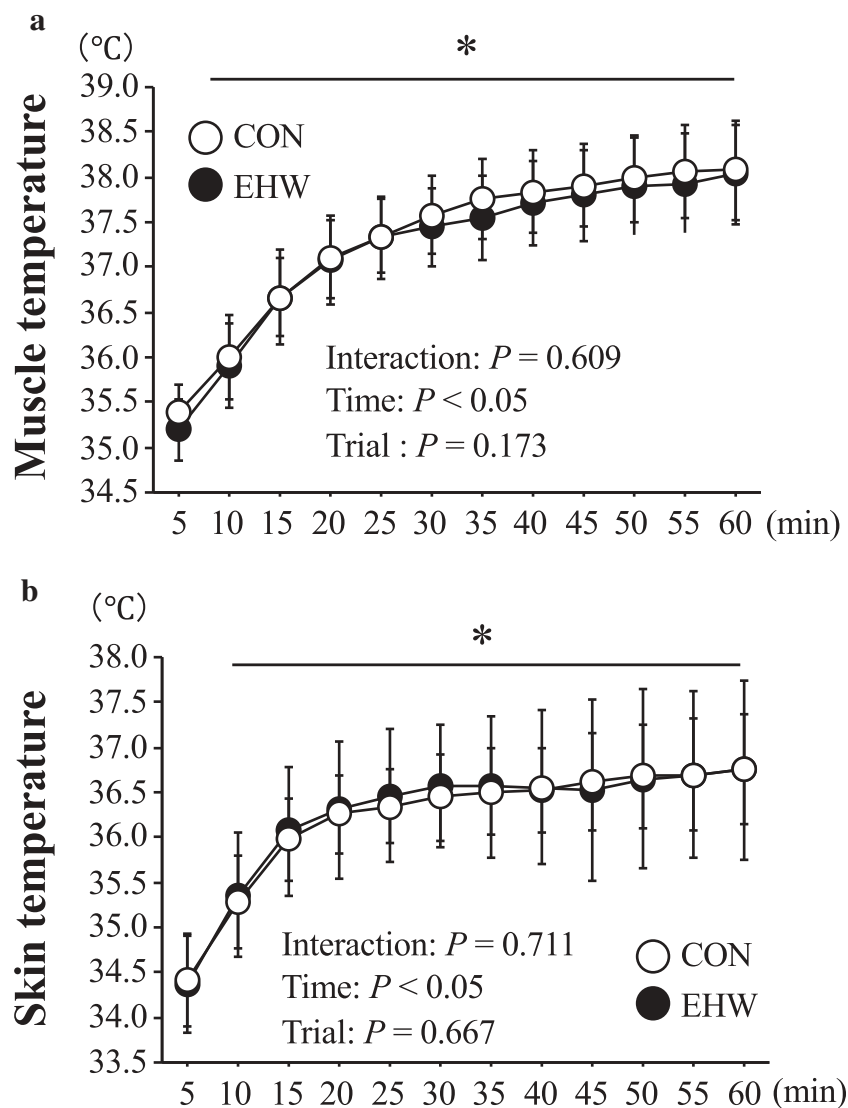


Figure 2. Tissue temperature during exercise. Values mean \pm SD ($n = 12$), *; $P < 0.05$ vs. Pre.

($P = 0.97$). The scores for RPE, thermal sensation, and stomach fullness significantly increased as the exercise progressed (main effect of time, $P < 0.01$). However, no significant differences were observed between the trials at any time point (interaction, $P > 0.05$, main effect of time, $P > 0.05$).

Respiratory variables

Figure 3 shows the changes in the respiratory variables during exercise. $\dot{V}O_2$ during exercise tended to be lower in the EHW trial (44.7 ± 4.4 mL/kg/min) than in the CON trial (46.1 ± 4.4 mL/kg/min, $P = 0.09$). Moreover, $\dot{V}CO_2$ was significantly lower in the EHW trial (40.1 ± 4.5 mL/kg/min) than in the

CON trial (41.7 ± 4.1 mL/kg/min, $P = 0.03$). Consequently, the EHW trial resulted in a significantly lower energy expenditure compared to the CON trial (13.2 ± 1.6 vs. 13.7 ± 1.5 kcal/min, respectively, $P = 0.04$). $\dot{V}E$ did not significantly differ between the two trials ($P = 0.14$).

Blood variables

Tables 2 and 3 show the changes in blood parameters. The blood lactate concentration significantly increased as the exercise progressed (main effect of time, $P = 0.01$). The blood levels of hemoglobin and hematocrit significantly increased with exercise (main effect of time:

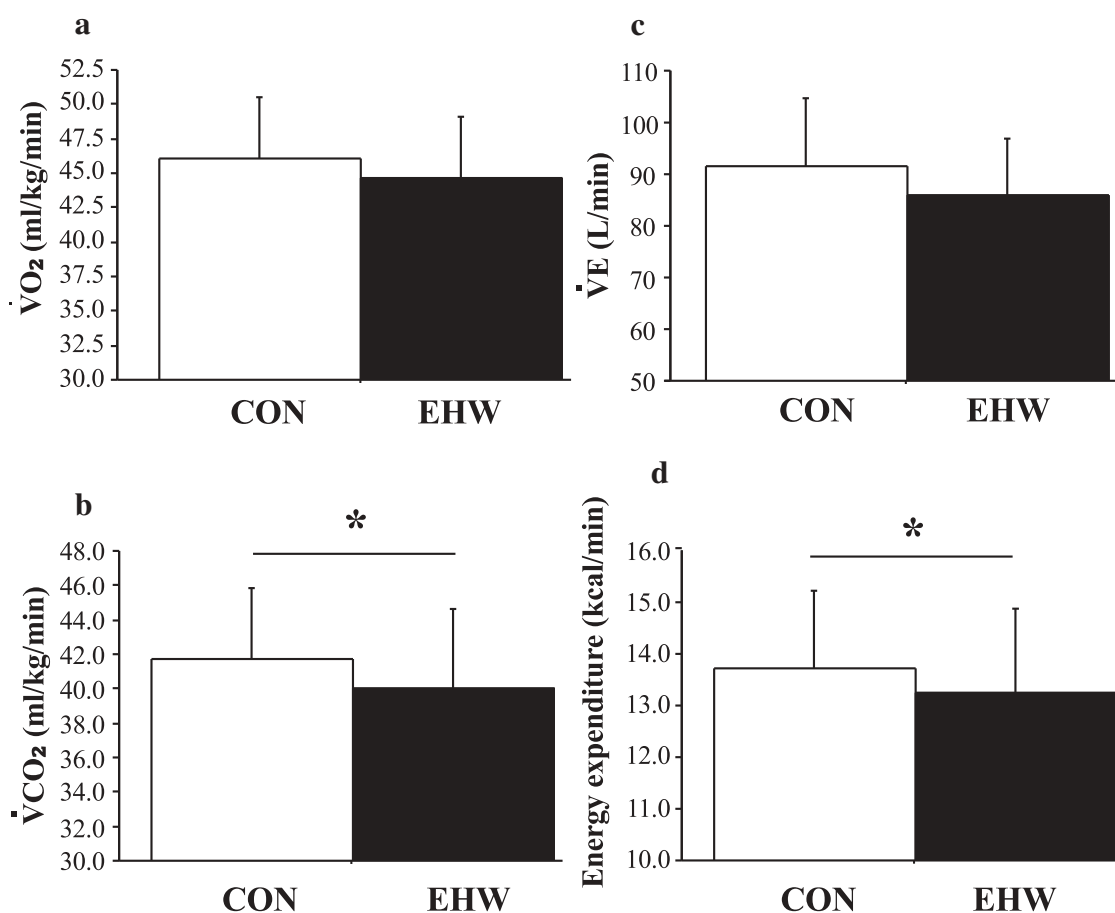


Figure 3. Respiratory variables during exercise. Values mean \pm SD ($n = 12$), *; $P < 0.05$ vs. Pre.

Table 2. Hemoglobin, hematocrit, serum osmolality levels, total protein and albumin concentrations.

		Pre	15 min	30 min	45 min	60 min	Post
Hemoglobin (g/dL)	CON	14.2 \pm 0.5	15.3 \pm 0.7*	15.4 \pm 0.6*	15.4 \pm 0.6*	15.5 \pm 0.6*	16.0 \pm 0.6*
	EHW	14.2 \pm 0.4	15.2 \pm 0.5*	15.5 \pm 0.5*	15.5 \pm 0.5*	15.1 \pm 1.5*	16.2 \pm 0.7*
Hematocrit (%)	CON	42.7 \pm 1.4	45.8 \pm 2.0*	46.2 \pm 1.7*	46.3 \pm 1.8*	46.5 \pm 1.9*	48.1 \pm 1.7*
	EHW	42.8 \pm 1.1	45.8 \pm 1.5*	46.6 \pm 1.5*	46.5 \pm 1.6*	45.3 \pm 4.5*	48.6 \pm 2.0*
Serum osmolality (mOsm/kg·H ₂ O)	CON	284 \pm 2.7	290 \pm 3.6*	291 \pm 4.2*	291 \pm 4.2*	292 \pm 4.4*	296 \pm 5.8*
	EHW	284 \pm 2.3	290 \pm 3.7*	290 \pm 3.1*	291 \pm 2.2*	292 \pm 3.1*	294 \pm 4.7*
Total protein (g/dL)	CON	7.2 \pm 0.3	7.0 \pm 0.3	7.0 \pm 0.3*	7.0 \pm 0.3*	7.0 \pm 0.3	6.2 \pm 2.0*
	EHW	7.2 \pm 0.2	7.1 \pm 0.3	7.0 \pm 0.3	7.0 \pm 0.3	7.2 \pm 0.5	6.8 \pm 0.3*
Albumin (g/dL)	CON	4.7 \pm 0.2	4.6 \pm 0.3	4.6 \pm 0.2	4.6 \pm 0.3	4.7 \pm 0.3	4.2 \pm 1.3*
	EHW	4.8 \pm 0.2	4.6 \pm 0.3	4.6 \pm 0.3	4.6 \pm 0.3	4.7 \pm 0.3	4.5 \pm 0.3*

Values are means \pm SD ($n = 12$); *; $P < 0.05$ vs Pre

$P < 0.01$). Moreover, the plasma volume (calculated from the hemoglobin and hematocrit [19]) significantly decreased during exercise ($P < 0.01$). However, these values during exercise did not significantly differ between the trials (interaction, $P = 0.77$; main effect of trial, $P = 0.61$, Figure 4). Blood pH and HCO₃⁻, Na⁺, and K⁺ levels significantly changed with exercise (main effect of time, $P < 0.01$). However, no significant

interaction or main effect of time was observed in any parameter. Serum total protein, albumin and osmolality significantly changed with exercise (main effect of time, $P < 0.05$ for all variables), but these responses did not significantly differ between the trials (interaction and main effect of trial, $P > 0.05$ for all variables).

Figure 5 shows the changes in the serum glycerol concentration. Exercise significantly

Table 3. Blood pH, HCO₃⁺, PO₂, PCO₂ levels, Na⁺ and K⁺ concentrations.

		Pre	15 min	30 min	45 min	60 min	Post
pH	CON	7.41 ± 0.02	7.41 ± 0.02	7.42 ± 0.02	7.45 ± 0.02*	7.45 ± 0.03*	7.39 ± 0.04*
	EHW	7.41 ± 0.01	7.41 ± 0.02	7.42 ± 0.02	7.44 ± 0.02*	7.44 ± 0.02*	7.40 ± 0.04*
HCO ₃ ⁺ (mmol/L)	CON	27.2 ± 0.2	21.2 ± 0.4*	20.3 ± 0.4*	20.0 ± 0.3*	20.2 ± 0.3*	14.3 ± 1.4*
	EHW	27.4 ± 0.3	21.6 ± 0.6*	20.9 ± 0.6*	20.4 ± 0.7*	21.3 ± 0.5*	16.4 ± 0.7*
PO ₂ (kPa)	CON	8.27 ± 2.19	9.98 ± 0.96*	10.11 ± 1.01*	9.92 ± 1.05*	9.83 ± 0.97*	8.89 ± 1.61*
	EHW	8.19 ± 1.83	9.2 ± 1.27*	9.13 ± 1.48*	9.35 ± 1.39*	9.39 ± 1.36*	9.15 ± 1.69*
PCO ₂ (kPa)	CON	5.85 ± 0.27	5.21 ± 0.24*	4.92 ± 0.27*	4.59 ± 0.35*	4.59 ± 0.32*	4.41 ± 0.3*
	EHW	5.91 ± 0.27	5.29 ± 0.43*	5.09 ± 0.44*	4.76 ± 0.51*	4.82 ± 0.31*	4.51 ± 0.45*
Na ⁺ (mmol/l)	CON	138.9 ± 1.2	123.7 ± 6.4	121.5 ± 4.5*	120.8 ± 5.5*	120 ± 5.5*	111.9 ± 4.8*
	EHW	139.0 ± 2.1	124.7 ± 6.0*	120.5 ± 6.3*	119.9 ± 6.3*	122.1 ± 8.7*	111.2 ± 6.8*
K ⁺ (mmol/L)	CON	3.65 ± 0.20	4.27 ± 0.22*	4.21 ± 0.14*	4.24 ± 0.19*	4.38 ± 0.3*	4.24 ± 1.32*
	EHW	3.70 ± 0.18	4.17 ± 0.27*	4.16 ± 0.33*	4.18 ± 0.33*	4.33 ± 0.44*	4.16 ± 0.39*

Values are means ± SD (n = 12) *; P < 0.05 vs Pre

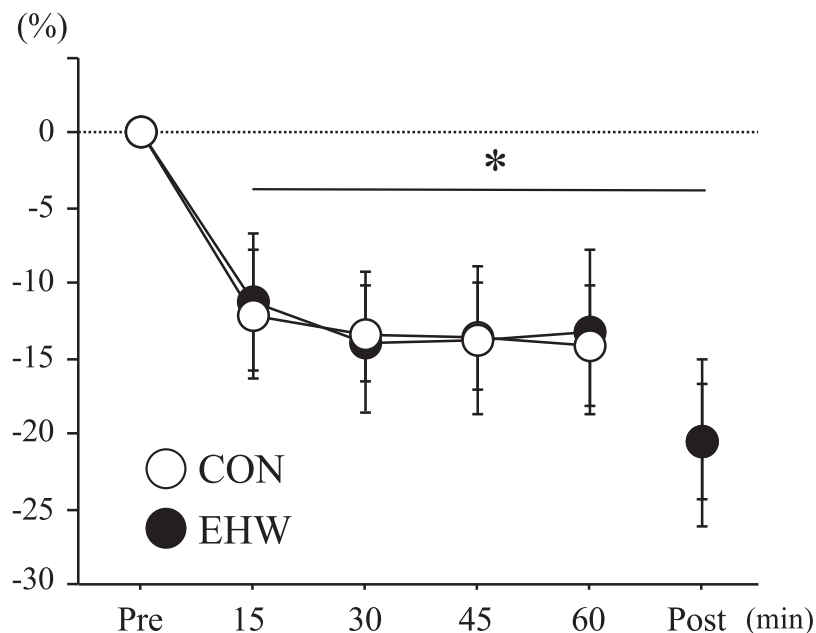


Figure 4. Plasma volume change during exercise. Values mean ± SD (n = 12), *; P < 0.05 vs. Pre.

increased serum glycerol concentration (main effect of time, $P < 0.05$), but no significant interaction ($P = 0.47$) was observed. Furthermore, the area under the curve during the 60 min exercise did not significantly differ ($P = 0.13$) between the EHW (15.7 ± 8.7 mg/dL·60 min) and CON (19.1 ± 16.0 mg/dL·60 min) trials.

Time to exhaustion during the incremental pedaling test

TTE during the incremental pedaling test did not significantly differ ($P = 0.25$) between the CON (350 ± 83 s) and EHW (312 ± 90 s) trials.

Discussion

Body fluid balance, energy metabolism

Our initial hypothesis was that drinking EHW would attenuate the disturbance in the body fluid balance (i.e., plasma volume loss, increased serum osmolality) during endurance exercise in a heated environment. Especially, we expected that EHW ingestion could attenuate the plasma volume loss during early phase of prolonged exercise due to rapid absorption after water ingestion. In a previous study, EHW ingestion attenuated blood viscosity after exercise-induced dehydration [10]. By contrast, we found no significant

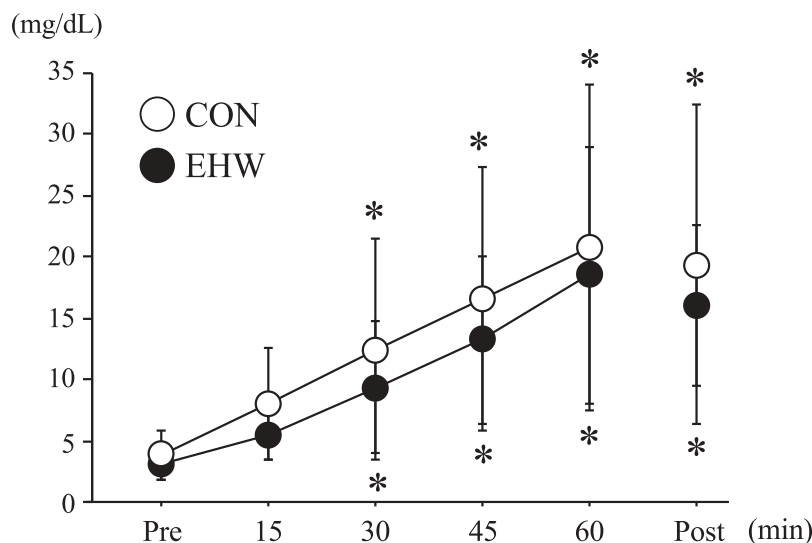


Figure 5. Serum glycerol concentration during exercise. Values mean \pm SD ($n = 12$), *; $P < 0.05$ vs. Pre.

difference in the plasma volume shift during endurance exercise between trials, suggesting that EHW ingestion during endurance exercise did not affect the exercise-induced plasma volume loss and shift in a heated environment. As a plausible reason for the inconsistent findings, Weidman et al. [10] determined blood viscosity during the rehydration period after exercise-induced dehydration in a warm environment using a different experimental design. Endurance exercise in a heated environment enhances the sweating rate and evaporation [3]. However, EHW ingestion appeared to be insufficient to attenuate the body fluid loss during exercise in a heated environment.

A novel finding of our study was significantly lower energy expenditure during the 60 min pedaling exercise in the EHW trial. Because all subjects completed both exercise trials using the same workload, the lower energy expenditure during exercise reflects the improved exercise tolerance with EHW ingestion during exercise in a heated environment. We feel that EHW ingestion may alter the substrate oxidation pattern during exercise. In an animal study, EHW (hydrogen rich water) ingestion augmented fat oxidation (Kamimura et al [20]). Furthermore, fat mass was reduced following hydrogen rich water ingestion in obese and type II diabetic mice., which may be associated with decreased oxidation stress and augmented fibroblast growth factor-21 (FGF21) [20]. However, fat oxidation (evaluated by RER) during exercise did not significantly differ between the trials. Additionally, the exercise-induced elevation of serum glycerol

concentrations (an indication of exercise-induced lipolysis) was not significantly different between the trials. Therefore, an altered substrate oxidation pattern was not involved in the lowered energy expenditure in the EHW trial. As another factor, improved fluid absorption in the gastrointestinal tract with concomitant attenuation of the plasma volume loss and HR elevation during exercise might be involved. These factors would decrease cardiovascular strain, leading to the reduction of energy expenditure during exercise in the EHW trial. However, as mentioned above, related parameters (i.e., plasma volume and HR) did not significantly differ between the two trials at any time point. Although the detailed reason for the lowered energy expenditure during endurance exercise in the EHW trial is not clear, the finding suggests the efficacy of EHW consumption during endurance exercise in a heated environment.

Acid–base balance

Ostojic et al. [12] reported that drinking hydrogen-rich water (2 L/day) for 14 days increased baseline blood pH. Therefore, ingesting hydrogen molecules may modify the acid–base balance (e.g., lowered blood pH) during exercise. However, the changes in blood parameters associated with the acid–base balance (e.g., blood pH, HCO_3^-) did not significantly differ between the two trials at any time point. There was a large difference in the experimental design between Ostojic et al. [12] and the present study, particularly the period of

EHW ingestion (14 days of ingestion vs. acute ingestion on the testing day). This difference may explain the inconsistent outcomes.

Exercise performance

Endurance exercise performance was assessed using the TTE during an incremental pedaling test following 60 min of endurance exercise. We hypothesized that ingesting EHW would prolong the TTE due to altered exercise-induced plasma volume loss. However, EHW ingestion did not affect the TTE during the incremental pedaling test. The energy expenditure during 60 min of continuous pedaling was significantly lower in the EHW trial. Theoretically, lower energy expenditure during submaximal endurance exercise would spare muscle glycogen and decrease cardiovascular strain, leading to improved exercise performance during the latter phase of the prolonged exercise [21]. However, the lower energy expenditure during 60 min of continuous exercise did not extend the TTE during the subsequent exercise, which lasted about 5 min. According to Jeukendrup et al. [21], saving 1% of the energy cost during submaximal cycling exercise improves performance during a 40 km cycling time trial [19]. Therefore, a potential reason for the lack of an apparent effect of the lower energy expenditure may be the exercise duration and situation. Furthermore, several studies have investigated the energy expenditure or energy cost during endurance exercise in real situations and have analyzed the correlation between exercise economy and performance variables (e.g., finishing time of competition) [22]. Cycling (running) economy is traditionally considered an important determinant of endurance exercise performance, although this has not been sufficiently confirmed in a laboratory setting [23]. Therefore, further determination on sports fields is required to draw robust conclusions.

There are several limitations to our study. All subjects drank either purified water or EHW on the experimental days only. Therefore, we cannot make a conclusion about the chronic effects of EHW ingestion. We also evaluated the body fluid balance based on the plasma volume shift and serum osmolality. However, we could not determine the absorption in the gastrointestinal tract. In a future study,

determining the gastric emptying rate may be valuable. Finally, the outcomes may depend on the dehydration level (about 1.7% for body weight in the present study). In bicycle road races, some athletes become severely dehydrated (e.g., 2.3% dehydration even when consuming 6 L of fluid) [24].

Conclusions

Drinking EHW during endurance exercise in a heated environment did not affect the exercise-induced acidification of blood, body fluid balance, or exercise performance. However, it lowered the energy expenditure during the 60 min pedaling exercise, suggesting the potential benefit of ingesting EHW during endurance exercise in the heat.

Acknowledgments

We greatly appreciate all subjects who conducted experimental trials in the heat environment. We also thank Dr. Kabayama for providing technical advice for preparing the EHW in this study.

Disclosure statement

An author (Kabayama S) is affiliated to NIHON TRIM CO., LTD, which sells a commercial generator of EHW. Although the author contributed to conducting the present experiment (e.g., preparing the generator, checking pH of generated water), he was not involved in manuscript writing, discussion or drawing conclusion, from the viewpoint of conflict of interest.

References

- [1] Kai Z, Dongke Z. Recent progress in alkaline water electrolysis for hydrogen production and applications. *Prog Energy Combust Sci.* 2010;36(3):307–326.
- [2] Xue J, Shang G, Tanaka Y, et al. Dose-dependent inhibition of gastric injury by hydrogen in alkaline electrolyzed drinking water. *BMC Compl Altern Med Mar.* 2014;14(1):81.
- [3] Gagnon D, Jay O, Kenny GP. The evaporative requirement for heat balance determines whole-body sweat rate during exercise under conditions permitting full evaporation. *J Physiol.* 2013;591(11):2925–2935.
- [4] Logan-Sprenger HM, Heigenhauser GJ, Jones GL, et al. The effect of dehydration on muscle metabolism and time trial performance during prolonged cycling in males. *Physiol Rep.* 2015;3(8):pii: e12483.
- [5] Buono MJ, Krippes T, Kolkhorst FW, et al. Increases in core temperature counterbalance effects of

- hemoconcentration on blood viscosity during prolonged exercise in the heat. *Exp Physiol.* **2016**;101(2):332–342.
- [6] González-Alonso J, Mora-Rodríguez R, Below PR, et al. Dehydration reduces cardiac output and increases systemic and cutaneous vascular resistance during exercise. *J Appl Physiol.* **1985**;79(5):1487–1496.
- [7] Kenefick RW, Sollanek KJ, Charkoudian N, et al. Impact of skin temperature and hydration on plasma volume responses during exercise. *J Appl Physiol.* **2014**;117(4):413–420.
- [8] Chevront SN, Kenefick RW, Montain SJ, et al. Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol.* **2010**;109(6). DOI:10.1152/jappphysiol.00367.2010
- [9] Sawka MN, Chevront SN, Kenefick RW. Hypohydration and human performance: impact of environment and physiological mechanisms. *Sports Med.* **2015**;45(Suppl, 1):51–60.
- [10] Weidman J, Holsworth RE Jr, Brossman B, et al. Effect of electrolyzed high-pH alkaline water on blood viscosity in healthy adults. *J Int Soc Sports Nutr.* **2016**;13:45.
- [11] Aoki K, Nakao A, Adachi T, et al. Pilot study: effects of drinking hydrogen-rich water on muscle fatigue caused by acute exercise in elite athletes. *Med Gas Res.* **2012**;2(1):12.
- [12] Ostojic SM, Stojanovic MD. Hydrogen-rich water affected blood alkalinity in physically active men. *Res Sports Med.* **2014**;22(1):49–60.
- [13] Metzger JM, Moss RL. Greater hydrogen ion-induced depression of tension and velocity in skinned single fibers of rat than slow muscles. *J Physiol.* **1987**;393:727–742.
- [14] Holland JJ, Skinner TL, Irwin CG, et al. The influence of drinking fluid on endurance cycling performance: a meta-analysis. *Sports Med.* **2017**;47(11):2269–2284.
- [15] Ramanathan NL. A new weighting system for mean surface temperature of the human body. *J Appl Physiol.* **1964**;19:531–533.
- [16] Weir JB. New new methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol.* **1949** Aug;109(1–2):1–9.
- [17] Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc.* **1982**;14(5):377–381.
- [18] Trecroci A, Formenti D, Ludwig N, et al. Bilateral asymmetry of skin temperature is not related to bilateral asymmetry of crank torque during an incremental cycling exercise to exhaustion. *PeerJ.* **2018**;1:6:e4438.
- [19] Jeukendrup AE, Craig NP, Hawley JA. The bioenergetics of world class cycling. *J Sci Med Sport.* **2000**;4:414–433.
- [20] Kamimura N, Nishimaki K, Ohsawa I, et al. Molecular hydrogen improves obesity and diabetes by inducing hepatic FGF21 and stimulating energy metabolism in db/db mice. *Obesity.* **2011**;7:1396–1403. DOI:10.1038/oby.2011.6
- [21] Burke LM, Hawley JA. Effects of short-term fat adaptation on metabolism and performance of prolonged exercise. *Med Sci Sports Exerc.* **2002**;34(9):1492–1498.
- [22] Bardis CN, Kavouras SA, Arnaoutis G, et al. Mild dehydration and cycling performance during 5-kilometer hill climbing. *J Athl Train.* **2013**;48(6):741–747.
- [23] Lundby C, Robach P. Performance Enhancement: what are the physiological limits? *Physiology (Bethesda).* **2015**;30(4):282–292.
- [24] Armstrong LE, Johnson EC, Kunces LJ, et al. Drinking to thirst versus drinking ad libitum during road cycling. *J Athl Train.* **2014**;49(5):624–631.